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PHYLOGENETIC ANALYSIS OF TRIBE SALSOLEAE (CHENOPODIACEAE) BASED ON RIBOSOMAL ITS SEQUENCES: IMPLICATIONS FOR THE EVOLUTION OF PHOTOSYNTHESIS TYPES[1]

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Diversity in photosynthetic pathways in the angiosperm family Chenopodiaceae is expressed in both biochemical and anatomical characters. To understand the evolution of photosynthetic diversity, we reconstructed the phylogeny of representative species of tribe Salsoleae of subfamily Salsoloideae, a group that exhibits in microcosm the patterns of photosynthetic variation present in the family as a whole, and examined the distribution of photosynthetic characters on the resulting phylogenetic tree. Phylogenetic relationships were inferred from parsimony analysis of nucleotide sequences of the internal transcribed spacer regions (ITS) of the 18S–26S nuclear ribosomal DNA of 34 species of *Salsola* and related genera (*Halothamnus, Climacoptera, Girgensohnia, Halocharis,* and *Haloxylon*) and representative outgroups from tribes Camphorosmeae (*Camphorosma lessingii, Kochia prostrata,* and *K. scoparia*) and Atripliceae (*Atriplex spongiosa*). A highly resolved strict consensus tree largely agrees with photosynthetic type and anatomy of leaves and cotyledons. The sequence data provide strong support for the origin and evolution of two main lineages of plants in tribe Salsoleae, with NAD-ME and NADP-ME C_4 photosynthesis, respectively. These groups have different C_4 photosynthetic types in leaves and different structural and photosynthetic characteristics in cotyledons. Phylogenetic relationships inferred from ITS sequences generally agree with classifications based on morphological data, but deviations from the existing taxonomy were also observed. The NAD-ME C_4 lineage contains species classified in sections *Caroxylon, Malpigipila, Cardiandra, Belanthera,* and *Coccosalsola,* and the NADP-ME lineage comprises species from sections *Coccosalsola* and *Salsola.* Reconstruction of photosynthetic characters on the ITS phylogeny indicates separate NAD-ME and NADP-ME lineages and suggests two reversions to C_3 photosynthesis. Reconstruction of geographic distributions suggests Salsoleae originated and diversified in central Asia and subsequently dispersed to Africa, Europe, and Mongolia. Inferred patterns and processes of photosynthetic evolution in Salsoleae should further our understanding of biochemical and anatomical evolution in Chenopodiaceae as a whole.

Key words: C_3 and C_4 photosynthesis; Chenopodiaceae; evolution; ITS sequences; leaf anatomy; phylogeny; *Salsola*.

The angiosperm family Chenopodiaceae exhibits great diversity in photosynthetic pathway and in the structure of the CO_2 assimilation organs. Both C_3 and C_4 photosynthesis are found in four tribes of the family: Atripliceae, Camphorosmeae, Suaedeae, and Salsoleae (Carolin, Jacobs, and Vesk, 1975; Shomer-Ilan, Nissenbaum, and Waisel, 1981; Zalenskii and Glagoleva, 1981; Voznesenskaya and Gamaley, 1986; Pyankov et al., 1992, 1997; Akhani, Trimborn, and Ziegler, 1997). Among the C_4 species, variation also occurs in the types of Kranz anatomy (details in Carolin, Jacobs, and Vesk, 1975) and in the biochemical subtypes of the C_4 pathway. This variation is generally correlated, such that species with Atriplicoid and Suaedoid types of Kranz anatomy have the NAD-ME C_4 photosynthetic subtype, and species with Kochioid anatomy have the NADP-ME C_4 photosynthetic subtype. Only the Salsoloid anatomical group has both NAD-ME and NADP-ME subtypes (Zalenskii and Glagoleva, 1981; Pyankov and Vakhrusheva, 1989; Gamaley et al., 1992; Pyankov et al., 1992, 1997; Fisher et al., 1997). The general correspondence of anatomical type and tribal limits inferred from morphology suggests multiple evolutionary lineages with C_4 photosynthesis (Pyankov, 1991; Gamaley et al., 1992; Pyankov et al., 1992, 1997), as in the Poaceae (Hattersley and Watson, 1992; Kellogg, 1999).

Diversity in habitats, life forms, and photosynthetic characters in assimilation organs is particularly complex in *Salsola,* a genus of 100 (Freitag, 1997) to nearly 200 (Botschantzev, 1967, 1968, 1969, 1979) species, and related genera in tribe Salsoleae. Two anatomical types, Salsoloid and Sympegmoid (Carolin, Jacobs, and Vesk, 1975), occur in leaves of species of *Salsola.* Salsoloid type leaves are characterized by two continuous layers of chlorenchymatous cells (a layer of palisade mesophyll cells and an inner layer of very distinctive Kranz type bundle cells) on the periphery and water-storage parenchyma in the center. The main vascular bundle occupies the central position in the leaf, and only the small, peripheral vascular bundles are in contact with the chlorenchyma. Some species with Salsoloid anatomy have NAD-ME C_4 photosynthesis whereas others have the NADP-ME C_4 subtype (Zalenskii and Glagoleva, 1981; Pyankov and Vakhrusheva, 1989; Pyankov et al., 1992, 1997). Sympegmoid type leaves are characterized by having two or three layers of palisade cells and a discontinuous layer of indistinctive bundle sheath cells (typically non-Kranz) around water-storage tissue (Carolin, Jacobs, and

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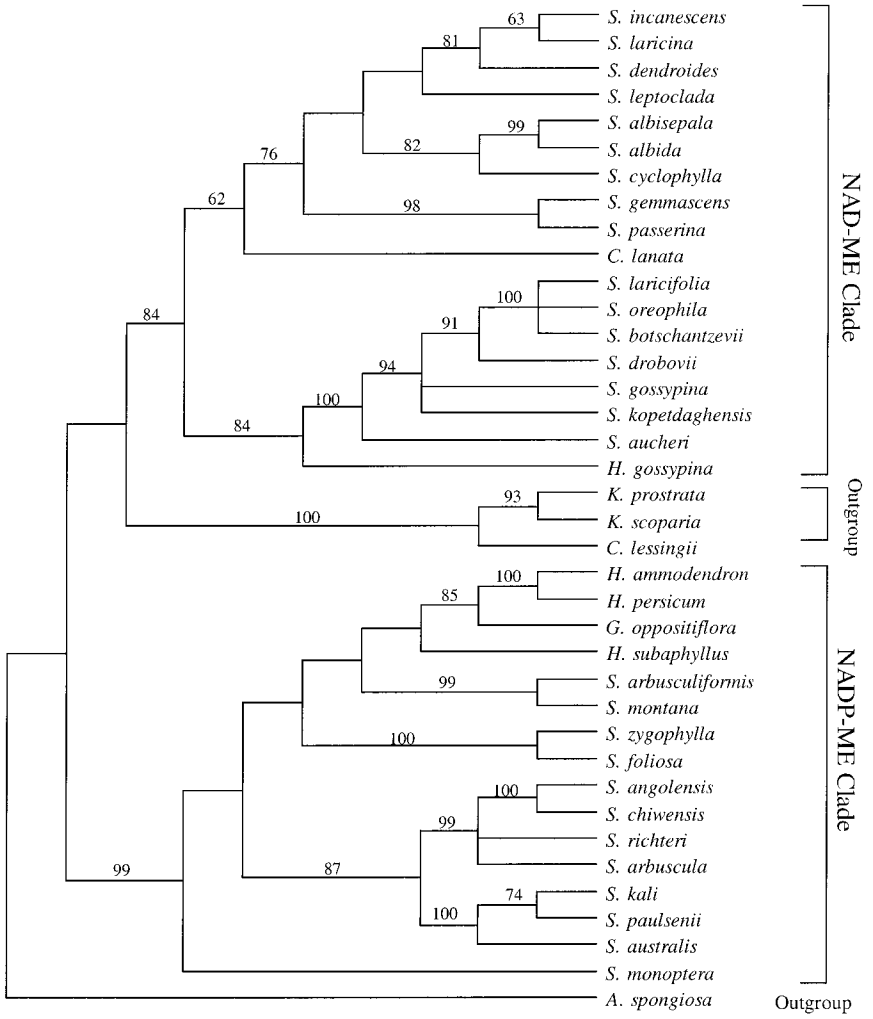

Fig. 1. Strict consensus of the 12 shortest trees found in parsimony searches of the ITS data set for Salsoleae and outgroups. Bootstrap values are given above branches.

Vesk, 1975; Pyankov et al., 1997). Plants with Sympegmoid anatomy have C_3-like $^{13}C/^{12}C$ carbon discrimination values (Akhani, Trimborn, and Ziegler, 1997; Pyankov et al., 1997). Variation also occurs in structural and biochemical features in cotyledons (Pyankov et al., 1998; Pyankov, Artyusheva, and Edwards, 1999). Two non-Kranz types, isopalisade and dorsoventral, and two types of Kranz anatomy, Atriplicoid and Salsoloid, are found in *Salsola* cotyledons (Butnik, 1979; Butnik et al., 1991; Pyankov et al., 1998; Pyankov, Artyusheva, and Edwards, 1999). Finally, Kranz-type cotyledons and leaves may or may not contain a hypodermis. The result is a number of unique combinations of structural and biochemical photosynthetic types in leaves and cotyledons in species of Salsoleae.

Kellogg (1999) demonstrated multiple origins of C_4 photosynthesis in each of the families Poaceae, Cyperaceae, Asteraceae, and Zygophyllaceae. Multiple origins of C_4 photosynthesis appear likely within Chenopodiaceae as well, and the diversity of photosynthetic types and anatomical structures in Salsoleae suggests a dynamic pattern of photosynthetic evolution within this single tribe. Despite extensive systematic treatments of *Salsola* and relatives (e.g., Botschantzev, 1969; Freitag, 1997), the number of species and species groups remains uncertain, and no explicit phylogeny of Salsoleae has been presented to place the photosynthetic diversity in a historical context.

The internal transcribed spacer (ITS) region of 18S–26S nuclear ribosomal DNA (nrDNA) has proven to be a useful source of characters for phylogenetic relationships within genera and among closely related genera in many angiosperm families (e.g., Baldwin et al., 1995). We therefore used ITS sequences to reconstruct the phylogeny of tribe Salsoleae in order to address questions about the origin and evolution of the C_4 syndrome in *Salsola* and related genera.

MATERIALS AND METHODS

Plant material and DNA extraction—Our sampling strategy for the phylogenetic analysis was (1) to include representative species having all combinations of photosynthetic structures and biochemistry that are present in Salsoleae and (2) to include representatives of all six currently recognized sections of *Salsola* (Botschantzev, 1969, 1979; Tzvelev, 1993; Freitag, 1997). Other genera of Salsoleae, considered by Botschantzev (1969) to be derived from *Salsola*, were also included. Four species from outside Salsoleae (*Camphorosma lessingii, Kochia prostrata, K. scoparia,* and *Atriplex spongiosa*) were included as outgroups (Table 1).

Total DNA was isolated from leaves of a single individual using a modified

CTAB buffer method (Doyle and Doyle, 1987) and a microprep procedure (Cullings, 1992). Both fresh leaves from living plants and dried leaves taken from herbarium specimens served as sources of DNA. Fresh leaves were desiccated on silica gel for 1 wk prior to DNA extraction.

DNA amplification and sequencing—The ITS regions were amplified using the primer combination N-nc18S10/C26A (Wen and Zimmer, 1996). Automated sequencing was performed using these same primers, following the general methods outlined by Soltis and Soltis (1997) and the Big Dye Deoxy Terminator (Applied Biosystems, Inc., Foster City, California, USA) with an ABI 377 automated DNA sequencer.

Alignment and phylogenetic analysis—ITS sequences were deposited in GenBank (accession numbers AF318619–AF318656). ITS sequences were aligned manually by sequential pairwise comparison. Gaps of one or more nucleotide positions were inserted to align the sequences; gaps were scored as missing data in phylogenetic analyses. Parsimony analyses were conducted using PAUP* 4.0 (Swofford, 1998) on Macintosh Power PC computers. Heuristic searches involved 500 replicates with random taxon addition and nearest neighbor interchange (NNI) branch swapping, saving ten trees per replicate. These 840 trees (of lengths 861–882) served as starting trees for further analyses using tree bisection reconnection (TBR) branch swapping and saving all most parsimonious trees. Bootstrap analysis (Felsenstein, 1985) with 500 replicates, each with random taxon addition and TBR branch swapping and saving ten trees per replicate, was used to assess support for clades. Species from tribe Camphorosmeae (*Camphorosma lessingii*, *Kochia scoparia*, and *K. prostrata*) and tribe Atripliceae (*Atriplex spongiosa*) were used as outgroups.

Reconstructing the history of photosynthetic, anatomical, and distributional characters—To investigate the evolutionary history of photosynthetic and anatomical characters in both cotyledons and leaves of Salsoleae, we mapped the photosynthetic and anatomical types on the strict consensus tree using parsimony optimization and the TRACE option of MacClade version 3.04 (Maddison and Maddison, 1992). Photosynthesis in cotyledons was scored as C_3 (0), C_4-NAD-ME (1), and C_4-NADP-ME (2). Anatomy in cotyledons was scored as dorsoventral (DV; 0), isopalisade (IP; 1), Atriplicoid with hypodermis (ATR+H; 2), Salsoloid with hypodermis (SALS+H; 3), and Salsoloid without hypodermis (SALS−H; 4). Photosynthesis in leaves was scored as C_3 (0), C_4-NAD-ME (1), and C_4-NADP-ME (2). Anatomy in leaves was scored as Salsoloid with hypodermis (SALS+H; 0), Salsoloid without hypodermis (SALS−H; 1), Sympegmoid (SYMP; 2), Atriplicoid with hypodermis (ATR+H; 3), and Kochioid (KOCH; 4). All photosynthetic and anatomical characters were treated as unordered, and data used in reconstructions are from Butnik (1979), Gamaley et al. (1992), and Pyankov et al. (1997, 1998, unpublished data).

To evaluate Botschantzev's (1969) hypotheses of the location of origin and subsequent diversification and migration of *Salsola*, we plotted the geographic distributions using the TRACE option of MacClade and the strict consensus tree. Geographic distributions for species of Salsoleae and outgroups included in the analysis were taken from Botschantzev (1969, 1976, 1979). Nine geographic areas were identified and coded as southern Africa (0), southwestern Africa (1), northern Africa (2), Middle East (3), central Asia—large (4), central Asia—small (5), Mongolia (6), China (7), and Europe (8). The geographic designations for each species are given in Table 1; several species were coded as polymorphic.

RESULTS

Phylogenetic relationships—The aligned ITS sequences of Salsoleae and outgroups were 568 bp, 293 of which were variable and 211 of which were parsimony-informative. Twelve minimum-length trees of 861 steps were generated from parsimony analysis of *Salsola* and related genera (consistency index [CI] = 0.561; retention index [RI] = 0.745). The strict consensus of these trees (Fig. 1) is highly resolved, with only three polytomies (all trichotomies), all of which appear near the tips of the branches. The species of Salsoleae sampled do not form a clade to the exclusion of all outgroup species included. When the tree is rooted with *Atriplex spongiosa*, all remaining species form a clade composed of two large subclades. In the first subclade, the outgroups *Kochia prostrata*, *K. scoparia*, and *Camphorosma lessingii* form a clade (with 100% bootstrap support) that is sister to a clade (with 84% bootstrap support) of 18 species of Salsoleae. However, the inclusion of *Kochia* and *Camphorosma* with these species of Salsoleae does not receive bootstrap support ≥50%. The outgroups *K. prostrata*, *K. scoparia*, and *C. lessingii* are NADP-ME type C_4 species. The clade of 18 species of Salsoleae includes 14 species with NAD-ME C_4 photosynthesis and four species with C_3 or C_3–C_4 photosynthesis, in leaves. The second subclade (with 99% bootstrap support) contains the remaining 16 species of Salsoleae, all but two (with C_3 or C_3–C_4-like photosynthesis) of which have NADP-ME C_4 photosynthesis in leaves.

Within the larger subclade of Salsoleae (labeled the NAD-ME clade in Fig. 1) are two main clades, supported by bootstrap values of 62 and 84%, respectively. Relationships within the smaller subclade of Salsoleae (labeled the NADP-ME clade in Fig. 1) are highly resolved in all shortest trees, but the basal relationships within this clade received bootstrap support <50%.

Both the NAD-ME and NADP-ME clades contain species assigned to other genera that were considered ''derivatives'' of *Salsola* by Botschantzev (1969). In the NAD-ME clade are *Climacoptera lanata* and *Halocharis gossypina*. The NADP-ME clade includes *Halothamnus subaphyllus*, *Haloxylon ammodendron*, *H. persicum*, and *Girgensohnia oppositiflora*.

Character reconstructions—Parsimony reconstructions of photosynthetic and anatomical characters show dynamic patterns of evolution in features of both cotyledons and leaves. The reconstruction of photosynthetic type in mature leaves on all trees supports the split into two groups in Salsoleae (the NAD-ME and NADP-ME lineages) (Fig. 2). Although the ancestral nodes are equivocal, reversion to C_3 (or C_3-like) photosynthesis in leaves occurred once in each lineage, in the ancestor of *S. drobovii*, *S. laricifolia*, *S. oreophila*, and *S. botschantzevii* in the NAD-ME lineage and in the ancestor of *S. montana* and *S. arbusculiformis* in the NADP-ME lineage. The ancestral leaf anatomy in Salsoleae was reconstructed on all trees as Salsoloid without hypodermis (SALS−H) (Fig. 3). All other types apparently evolved from SALS−H. The addition of hypodermis (SALS+H) and Sympegmoid anatomy both arose independently in the NAD-ME and NADP-ME lineages from SALS−H ancestors.

Optimization of photosynthetic types in cotyledons on the strict consensus tree shows the ancestral condition in Salsoleae plus *Kochia* and *Camphorosma* (of tribe Camphorosmeae) to be equivocal. C_4 NADP-ME photosynthesis is present in both the Camphorosmeae and the NADP-ME lineage of Salsoleae. C_4-NAD-ME photosynthesis is present in the NAD-ME lineage, and C_3 photosynthesis is present in both the NAD-ME and NADP-ME lineages (Fig. 4). The history of cotyledon anatomy is also equivocal on the basal branches (Fig. 5).

Optimization of geographic distributions on the strict consensus tree reconstructs an origin for Salsoleae in central Asia (Fig. 6). From central Asia, species of Salsoleae dispersed to Europe (e.g., *S. kali*), Africa, and Mongolia. Migration to Africa appears to have occurred multiple times to account for the

TABLE 1. Species examined for nucleotide variation in ITS sequences, and the life form, geographic distribution, geographic origin of sample, and anatomy and biochemistry of each. ND = no data.

Species[a]	Form[b]	Geographic distribution[c]	Sample origin[d]	Cotyledons[e]		Leaves[e]	
				Mesophyll type	Biochemistry type	Mesophyll type	Biochemistry type
Tribe Atripliceae							
Atriplex spongiosa F. Muell	ANN	Australia	AUSTR	ND	ND	C4-ATR(+H) [A-7]	NAD [E-8]
Tribe Camphorosmeae							
Kochia prostrata (L.) Schard.	SS	M East, C Asia and S Europe; 4, 8	UZB-1	C4-ATR(+H) [A-1, 9]	NADP [E-9]	C4-KOCH(+H) [A-1, 9]	NADP [C-12; E-2, 9; P-2, 6]
Kochia scoparia (L.) Schrad.	ANN	M East, C Asia and S Europe; 4, 8	UZB-1	C4-ATR(+H) [A-4]	NADP [P-4]	C4-KOCH(+H) [A-1, 4]	NADP [P-4; E-4]
Camphorosma lessingii Litv.	SS	M East, C Asia and S Europe; 4, 8	UZB-1	C4-ATR(+H) [9]	NADP [E-9]	C4-KOCH(+H) [A-1, 2, 9]	NADP [E-2, 9; P-2; C-12]
Tribe Salsoleae							
Sect. *Caroxylon*							
S/s. *Caroxylon*							
S. albisepala Aellen	SH	S-W Africa; 1	HKBI	ND	ND	ND	C4 [C-12]
S. albida Botsch.	SH	S and S-W Africa; 0, 1	HKBI	ND	ND	ND	C4 [C-12]
S. angolensis Botsch.	SH	S-W Africa, Angola; 1	HKBI	ND	ND	ND	C4 [C-12]
S. cyclophylla Baker	SH	M East, Arabian Peninsula; 3	TURKM	ND	ND	ND	C4 [C-12]
S. dendroides Pall.	SH	M East, C Asia, Caucuses; 4	TURKM	C4-ATR(+H) [A-1, 9]	NAD [E-9; P-9]	C4-SALS(+H) [A-1, 2, 9]	NAD [C-12; P-2, 9; E-2, 9]
S/s. *Vermiculatae*							
S. incanescens C.A. Mey.	ANN	M East, C Asia; 4	TURKM	C4-ATR(+H) [A-9]	NAD [C-9; E-9; P-9]	C4-SALS(+H) [A-9]	NAD [C-12; E-9; P-9]
S. laricina Pall.	SS	C Asia, S Russia, Caucasus; 4	UZB-1	C4-ATR(+H) [A-3]	NAD [C-3; E-3; P-3]	C4-SALS(+H) [A-3]	NAD [C-3; E-3; P-3]
Sect. *Malpigipila*							
S. gemmascens Pall.	SS	N Africa, C Asia, Mediterranean; 2, 4	UZB-2	DV [A-1, 3]	C3 [C-3]	C4-SALS(−H) [A-1, 2, 3]	NAD [C-2, 3; E-2, 3; P-2, 3]
S. passerina Bunge	SS	Mongolia; 6	MONG	ND	ND	ND	NAD [P-9]
Sect. *Cardiandra*							
S. leptoclada Gand.	ANN	M East, C Asia; 4	TURKM	DV [A-9]	C3	C4-SALS(−H) [A-9]	NAD [P-9]
Sect. *Belanthera*							
S. aucheri (Moq.) Bunge ex lljin	SS	N Iran, Turkmenistan; 5	TURKM	ND	ND	ND	C4 [C-12]
S. gossypina Bunge	ANN	M East, C Asia; 4	TURKM	DV [A-9]	C3	C4-SALS(−H) [A-9]	NAD [C-12; E-9; P-9]
S. kopetdaghensis (Botsch.) Botsch.	SS	M East, C Asia; 4	TURKM	DV [A-9]	C3	C4-SALS(−H) [A-9]	NAD [C-12; E-9; P-9]
Climacoptera lanata (Pall.) Botsch.	ANN	M East, C Asia; 4	UZB-3	DV [A-1]	C3 [C-12]	C4-SALS(−H) [A-1, 2, 6]	NAD [C-12; E-2, 9; P-2, 9]
Halocharis gossypina Korov. & Kinzikaeva	ANN	C Asia; 5	TURKM	DV [A-9]	C3 [C-12]	C4-SALS(−H) [A-9]	NAD [E-9; P-9]
Sect. *Coccosalsola*							
S/s. Arbuscula							
S. arbusculiformis Drob.	SH	M East, C Asia; 4	UZB-3	IP [A-9]	C3 [C-12]	SYMP [A-1, 2]	C3-C4 [C-2; A-2; E-9; P-9]
S. botschantzevii Kurbanov			HKBI	ND	ND	ND	C3 [C-12]
S. drobovii Botsch.	SS	C Asia; 5	HKBI	IP [A-1]	C3	SYMP [A-1, 2]	C3 [C-12]
S. laricifolia Turcz. & Litv.	SH	C Asia, N-W China, Mongolia; 5, 6, 7	HKBI	ND	ND	ND	C3 [C-12]
S. montana Litv.	SS	C Asia; 5	HKBI	IP [A-1]	C3	SYMP [A-1, 2]	C3 [C-2]
S. oreophila Botsch.	SS	M East, C Asia; 4	HKBI	ND	ND	SYMP [A-1, 2]	C3 [C-2]

TABLE 1. Continued.

Species[a]	Form[b]	Geographic distribution[c]	Sample origin[d]	Cotyledons[e]		Leaves[e]	
				Mesophyll type	Biochemistry type	Mesophyll type	Biochemistry type
S. arbuscula Pall.	SH	M East, C Asia, S Russia, N-W China, Mongolia; 4, 6, 7	UZB-2	C4-SALS(+H) [A-1]	NADP [C-12; E-9; P-9]	C4-SALS(+H) [A-1, 2]	NADP [C-2; E-2; P-2]
S. chiwensis M. Pop.	SS	C Asia; 5	HKBI	C4-SALS(+H) [A-1]	C3	C4-SALS(+H) [A-1]	C4 [C-12]
S. richteri (Moq.) Kar. ex Litv.	SH	M East, C Asia; 4	TURKM	C4-SALS(+H) [A-1, 3]	NADP [C-3]	C4-SALS(+H) [A-1, 2, 3]	NADP [C-3; E-3; P-3, 6]
Halothamnus subaphyllus (C.A. Mey.) Botsch.	SH	C Asia; 5	UZB-1	IP [A-1, 2]	C3 [C-12]	C4-SALS(−H) [A-1, 2]	NADP [C-12; E-2; P-2]
Haloxylon ammodendron (C.A. Mey.) Bunge	SH	C Asia, N-W China; 5, 7	MONG	IP [A-9]	C3 [C-12]	C4-SALS(+H) [A-6]	NADP [C-5; E-5; P-5]
H. persicum Bunge ex Boiss. & Buhse	SH	M East, Central Asia, Arabian Peninsula; 4	UZB-2	IP [A-1, 5]	C3 [C-5]	C4-SALS(+H) [A-1, 2, 5]	NADP [C-5; E-2, 5; P-2, 5]
S/s. *Coccosalsola*							
S. zygophylla Batt. et Trab.	SH	N Africa, Algeria; 2	HKBI	ND	ND	ND	C4 (C-12)
S. foliosa (L.) Schrad.	ANN	C Asia, S Russia, N-W China, Mongolia; 4, 6, 7	HKBI	C4-SALS(+H) [A-1]	ND	C4-SALS(−H) [A-1]	C4 [C-12]
Sect. *Salsola*							
S. kali L.	ANN	S & W Europe; 8	EU	C4-SALS(−H) [A-11]	ND	C4-SALS(−H) [A-11]	NADP [C-12; E-10]
S. monoptera Bunge	ANN	S Russia, Altai; Mongolia; 6	HKBI	ND	ND	ND	C4 [C-12]
S. paulsenii Litv.	ANN	C Asia, S Russia, N-W China, Mongolia; 4, 6, 7	UZB-1	C4-SALS (−H) [A-1, 3]	NADP [C-3; E-3; P-3]	C4-SALS(−H) [A-1, 2]	NADP [C-2; E-2; P-2, 6]
S. australis R. Br.	ANN	M East, C Asia, Caucasus, S Europe, S Russia; 4, 8	UZB-1	C4-SALS(−H) [A-4]	NADP [E-4, P-4]	C4-SALS(−H) [A-4]	NADP [C-12; E-4; P-4, 6]
Girgensohnia oppositiflora (Pall.) Fenzl	ANN	C Asia, S Russia; 4	UZB-1	IP [A-9]	C3	C4-SALS(+H) [A-2]	NADP [E-2; P-2]

[a] Systematic subdivision, life forms, and geographic distribution of *Salsola* species by Botchantzev (1968, 1969, 1972, 1975, 1976, 1979). Names of species are according to Czerepanov (1995), except for *S. albisepala, S. zygophylla,* and *S. cyclophylla* from Botschantzev (1969) and *S. albida* and *S. angolensis* from Botchantzev (1974).

[b] ANN, annuals; SH, shrubs; SS, semi-shrubs.

[c] C, central; E, east; M, middle; N, north; S, south; W, west.

[d] AUSTR, Australia; EU, Europe; MONG, Mongolia, Trans-Altai Gobi desert; TURKM, Turkmenistan, Kopet-Dag hills, and Central Karakum desert; UZB, Uzbekistan; UZB-1, Uzbekistan, Samarkand region, near Nurata; UZB-2, Uzbekistan, Bukhara region, near Bukhara; UZB-3, South-West Kyzylkum desert, Uzbekistan, Bukhara region, near Dzhangeldy. Samples were collected from plants grown in greenhouse at Washington State University, Pullman, or field station at Urals State University, Ekaterinburg, Russia. HKBI indicates that dry samples from Herbarium of Komarov Botanical Institute, Saint-Petersburg, Russia, were used.

[e] Methods of identification of mesophyll anatomy and biochemical type: A, anatomy; C, $\delta^{13}C$ discrimination values; E, enzyme activities; P, primary photosynthetic products after 10 s fixation $^{14}CO_2$. Sources of data: *See following references and citations therein* 1, Butnik (1979); 2, Pyankov et al. (1997); 3, Pyankov et al. (1998); 4, Pyankov, Artyusheva, and Edwards (1999); 5, Pyankov et al. (1999); 6, Gamaley et al. (1992); 7, Osmond (1971); 8, Hatch and Kagawa (1974); 9, authors' unpublished data; 10, Gutierrez et al. (1974); 11, Olesen et al. (1974); 12, from carbon isotope analysis, C. C. Black and V. Pyankov, unpublished data. Abbreviations of mesophyll anatomy type and biochemistry type are described in the text.

distributions of the *S. albisepala-S. albida* clade, *S. zygophylla,* and *S. angolensis,* respectively. Likewise, migrations to Mongolia also apparently occurred multiple times: once for *S. gemmascens* and *S. passerina* and once for *S. monoptera,* plus independently in the broad-ranging *S. laricifolia, S. arbuscula, S. paulsenii, S. foliosa,* and *H. ammodendron.*

DISCUSSION

Phylogenetic analysis of ITS sequences provides strong evidence for the existence of two lineages within Salsoleae that correspond to NAD-ME and NADP-ME C_4 subtypes in leaves. These clades, reconstructed using DNA sequences, group species with common physiological features and photosynthetic characters in leaves and cotyledons (Pyankov et al., 1997, 1998; Pyankov, unpublished data). The ITS phylogeny also recovered groups of species that generally correspond to the sections and subsections of *Salsola* sensu Botschantzev (1969, 1976, 1979). The phylogeny also supports many of Botschantzev's (1969) hypotheses regarding the derivation of taxonomically recognized genera from within *Salsola* (e.g., *Halothamnus, Haloxylon, Girgensohnia, Climacoptera,* and *Halocharis*).

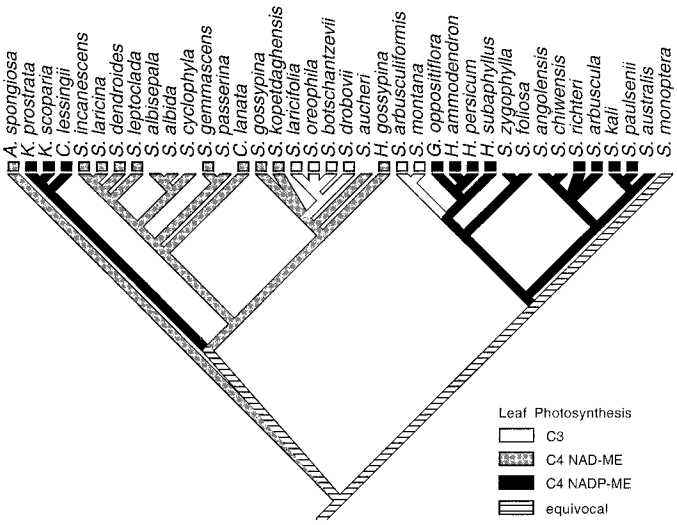

Fig. 2. Parsimony reconstruction of photosynthetic types in leaves on the strict consensus of the 12 most parsimonious ITS trees. The absence of a character-state ''box'' at the tip of a branch indicates that no data are available for that species.

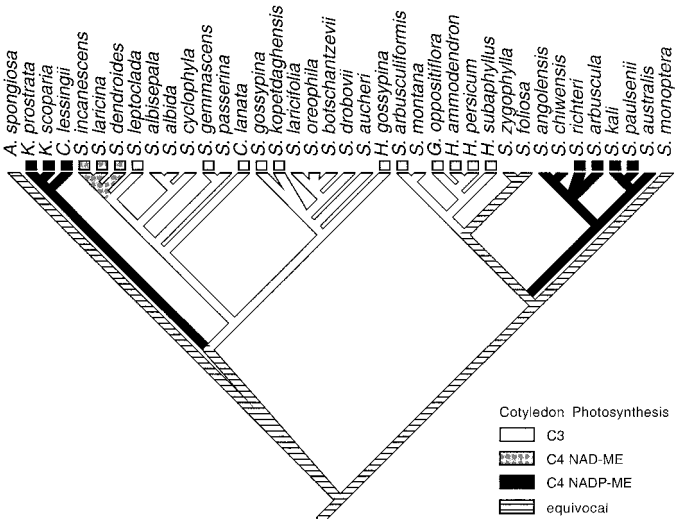

Fig. 4. Parsimony reconstruction of photosynthetic types in cotyledons on the strict consensus of the 12 most parsimonious ITS trees. The absence of a character-state ''box'' at the tip of a branch indicates that no data are available for that species.

Phylogeny: Comparisons with taxonomy and photosynthetic data*—NAD-ME lineage*—This lineage includes representatives of five sections, *Coccosalsola, Caroxylon, Malpigipila, Cardiandra,* and *Belanthera*; the latter three sections were considered by Botschantzev (1969) to have had a common origin from section *Caroxylon,* and other authors agreed regarding the morphological similarity and apparent close relationships of all of these sections except *Coccosalsola* (Tzvelev, 1993; Freitag, 1997).

Sister to the NAD-ME lineage are two of the outgroups, *Kochia* and *Camphorosma,* but this relationship is only weakly supported (bootstrap value <50%). The nonmonophyly of Salsoleae found here may be an artifact of the sampling used in this analysis or it may reflect true relationships in Chenopodiaceae. Ongoing revisionary work in Salsoleae is consistent with the hypothesis that *Salsola* is polyphyletic (H. Freitag, personal communication). Recent phylogenetic analyses have likewise demonstrated that Chenopodiaceae are not monophyletic, with genera of Amaranthaceae intermingled with those of Chenopodiaceae (e.g., Manhart and Rettig, 1994; Downie, Katz-Downie, and Cho, 1997). Further study is needed to test the monophyly of *Salsola* and of Salsoleae, to infer relationships among genera currently classified in Chenopodiaceae and Amaranthaceae, and to assess the evolution of photosynthetic characters in the Chenopodiaceae/Amaranthaceae clade.

Within the NAD-ME lineage of Salsoleae are two main clades. One of these consists of *Climacoptera lanata* as sister to a clade of species from sections *Cardiandra* (*S. leptoclada*),

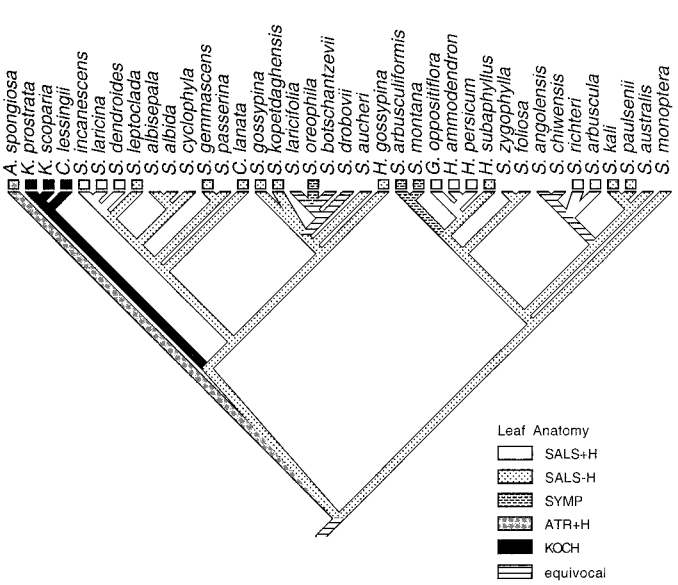

Fig. 3. Parsimony reconstruction of leaf anatomy on the strict consensus of the 12 most parsimonious ITS trees. The absence of a character-state ''box'' at the tip of a branch indicates that no data are available for that species.

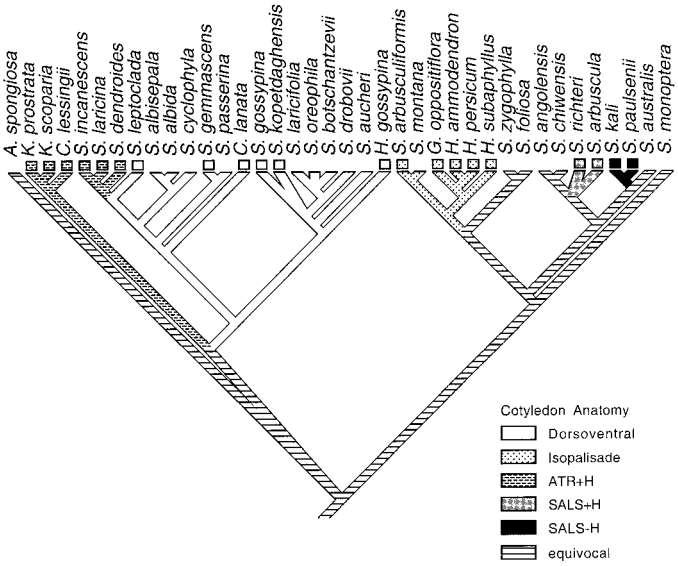

Fig. 5. Parsimony reconstruction of cotyledon anatomy on the strict consensus of the 12 most parsimonious ITS trees. The absence of a character-state ''box'' at the tip of a branch indicates that no data are available for that species.

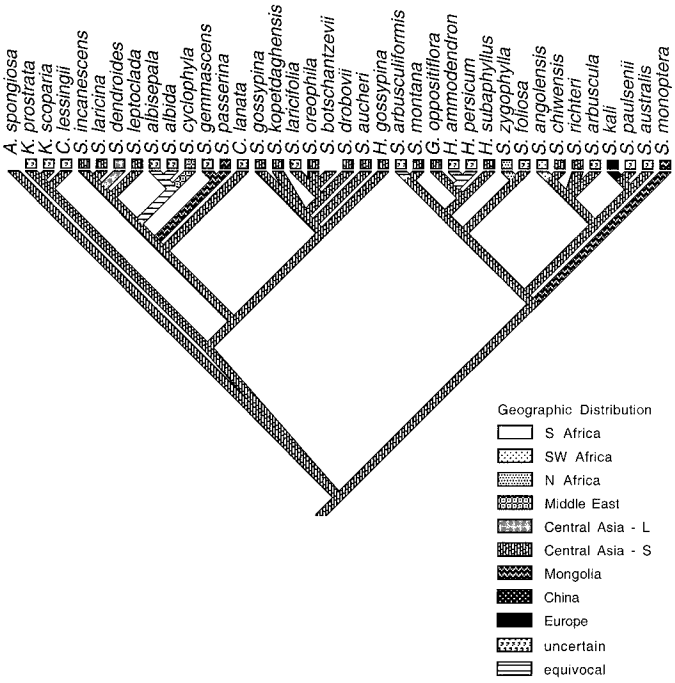

Fig. 6. Parsimony reconstruction of geographic distribution on the strict consensus of the 12 most parsimonious ITS trees. The absence of a character-state ''box'' at the tip of a branch indicates that no data are available for that species.

Malpigipila (*S. gemmascens* and *S. passerina*), and *Caroxylon* (six species). Three small clades within this clade received moderate to high bootstrap support: the sister relationship of *S. gemmascens* and *S. passerina* (98%), the *Caroxylon*-1 clade (*S. incanescens, S. laricina,* and *S. dendroides*; 81%), and the *Caroxylon*-2 clade (*S. cyclophylla, S. albisepala,* and *S. albida*; 82%). The *Caroxylon*-2 clade largely corresponds to Botschantzev's (1969) section *Caroxylon* subsection *Caroxylon,* although *S. angolensis* (which appears in the NADP-ME lineage) and *S. dendroides* (which is part of *Caroxylon*-1) are not included in the clade. *Salsola laricina* and *S. incanescens* belong to Botschantzev's (1969, 1979) subsection *Vermiculatae* and were considered to be derivatives of subsection *Caroxylon.* Freitag (1997) placed *S. dendroides* in subsection *Vermiculatae* as well, because it shares a hypogynous disc, linear filaments, and other characters with species in that subsection; this relationship to other species classified as subsection *Vermiculatae* is supported by the ITS phylogeny. All representatives of section *Caroxylon* examined have a similar type of Kranz anatomy in leaves (SALS+H) and cotyledons (ATR+H) (Table 1).

The *Malpigipila* clade (the sister pair of *S. gemmascens* and *S. passerina*) and *S. leptoclada* from section *Cardiandra* share similar features of photosynthesis in leaves and cotyledons. The cotyledons have C_3 type photosynthesis with dorsoventral mesophyll structure, and the leaves have C_4 NAD-ME type photosynthesis with Salsoloid Kranz anatomy without a hypodermis. Botschantzev (1969) proposed that section *Malpigipila* originated from section *Caroxylon* and that section *Cardiandra* is derived from *Malpigipila.* Although the relationships of these species are not well supported in the ITS phylogeny, the positions of species classified in sections *Cardiandra* and *Malpigipila* do not support Botschantzev's hypothesis.

The second large clade of the NAD-ME lineage includes *Halocharis gossypina* as sister to a clade of species from section *Belanthera* and section *Coccosalsola* subsection *Arbuscula* (with 94% bootstrap support). *Salsola aucheri, S. gossypina,* and *S. kopetdaghensis* of section *Belanthera* are successive sisters to a clade (91% bootstrap support) containing some, but not all, of Botschantzev's (1969) section *Coccosalsola* subsection *Arbuscula.* Included in this clade are *S. drobovii, S. laricifolia, S. oreophila,* and *S. botschantzevii. Salsola montana, S. arbusculiformis, S. arbuscula, S. chiwensis,* and *S. richteri,* all of which were also placed in subsection *Arbuscula* by Botschantzev, occur in the NADP-ME lineage. Botschantzev (1969) and others (Tzvelev, 1993; Freitag, 1997) considered sect. *Belanthera* to be derived from section *Caroxylon,* but this relationship is not supported by the ITS phylogeny (Fig. 1). Instead, the ITS phylogeny indicates a common ancestor for the *Belanthera* + *Coccosalsola* clade and the clade composed of species from sections *Caroxylon, Malpigipila, Cardiandra,* and *Coccosalsola. Climacoptera lanata* and *Halocharis gossypina* were considered by Botschantzev (1969) to be derived from section *Belanthera.* Given their phylogenetic position deep within *Salsola, Climacoptera* and *Halocharis* should be reclassified as species of *Salsola. Salsola aucheri, S. gossypina, S. kopetdaghensis,* and *H. gossypina* have the same photosynthetic structures and biochemistry as species of sections *Malpigipila* and *Cardiandra* (Table 1). Given the shared photosynthetic characteristics of most of the species in this clade, the common ancestor of the NAD-ME lineage had C_3 photosynthesis and dorsoventral anatomy in cotyledons and NAD-ME C_4 photosynthesis and SALS−H anatomy in leaves (Figs. 2–5). Species having C_4 type cotyledons evolved from C_3 in the NAD-ME lineage, but the results with species in the NADP-ME lineage are equivocal. However, the clade of *S. drobovii, S. laricifolia, S. oreophila,* and *S. botschantzevii* is characterized, in contrast, by leaves having Sympegmoid anatomy and C_3 (or C_3-like) photosynthesis based on carbon isotope composition (Butnik, 1979; Pyankov et al., 1997, unpublished data; see Table 1) and cotyledons having C_3 type anatomy (*S. drobovii,* information not available on other species). These characteristics indicate a derivation of Sympegmoid anatomy and reversion to C_3 photosynthesis in leaves in the common ancestor of this clade (Figs. 2, 3).

NADP-ME lineage—An independent evolutionary lineage with NADP-ME C_4 photosynthesis is strongly supported by both ITS sequence analysis (Fig. 1) and physiological data (Table 1). All species belonging to this lineage have different photosynthetic features in the assimilating organs than those in the NAD-ME line. All but two of them (which are C_3 or C_3-like) have NADP-ME C_4 photosynthesis in leaves; all have either NADP-ME C_4 photosynthesis in cotyledons with Salsoloid anatomy or C_3 photosynthesis with isopalisade mesophyll structure. Neither NAD-ME C_4 photosynthesis nor Atriplicoid or dorsoventral mesophyll types were found in species of this lineage. The species in the NADP-ME lineage belong to section *Coccosalsola* subsections *Arbuscula, Coccosalsola,* and *Genistoides* sensu Botschantzev (1976; equivalent to sections *Arbuscula, Coccosalsola,* and *Genistoides* sensu Freitag, 1997). Five small, well-supported clades are present within the NADP-ME lineage; relationships among these clades are completely resolved in all of the shortest trees, but they are not

supported by bootstrap values ≥50%. These five clades within the NADP-ME lineage will be discussed below.

1) *Sympegmoid (SYMP) clade*—Two species in our analysis, *S. arbusculiformis* and *S. montana,* form a sister pair in the NADP-ME lineage, with Sympegmoid anatomy. Although *S. arbusculiformis* has C_3-like $^{13}C/^{12}C$ carbon discrimination values, it resembles C_4 plants in its Kranz-like cells with many chloroplasts, indicative of a C_3–C_4 intermediate (Pyankov et al., 1997). Carolin, Jacobs, and Vesk (1975) suggested that Sympegmoid anatomy may have evolved from the Salsoloid type, a change that would require a reversion from C_4 to C_3 photosynthesis. The vasculature in the Sympegmoid type is very similar to that of Salsoloid Kranz anatomy, and it is unlike any other non-Kranz type in Chenopodiaceae. The presence of small vascular bundles just beneath the chlorenchymatous mesophyll further suggests a relationship with the Salsoloid type of Kranz anatomy. Sympegmoid leaves have three layers of chlorenchema cells, while Salsoloid leaves have two layers that surround a central bundle embedded in water storage tissue. Photosynthetically, *Salsola* species with the Sympegmoid structure belong to a group of plants classified as C_3, or C_3–C_4 intermediate species, which may represent an intermediate stage of evolution from C_3 to C_4 or vice versa (Edwards and Ku, 1987). The ITS phylogeny of Salsoleae suggests that species in the NADP-ME lineage with Sympegmoid anatomy (*S. arbusculiformis* and *S. montana*) evolved from *Salsola* species having C_4 photosynthesis. Several species of *Salsola* with Sympegmoid anatomy occur in the Pamir and Tien-Shan mountains; many C_4 *Salsola* species cannot survive in cooler conditions and may have disappeared during geological changes (Botschantzev, 1969). However, a reversion from C_4 to C_3 photosynthesis in these cool habitats seems likely (Pyankov et al., 1997) and is supported by the ITS phylogeny. Such a reversion from C_4 to C_3 photosynthesis has been inferred for *Eragrostis walteri,* the only species in *Eragrostis* that is not C_4 (Ellis, 1984).

As noted earlier, four species of the NAD-ME lineage (*S. drobovii, S. laricifolia, S. oreophila,* and *S. botschantzevii*) also have Sympegmoid anatomy and C_3-like photosynthesis, and Botschantzev (1969) placed these species, plus *S. montana, S. arbusculiformis, S. arbuscula, S. chiwensis,* and *S. richteri,* in section *Coccosalsola* subsection *Arbuscula.* However, reconstructions of anatomical and photosynthetic characters on the ITS tree show independent origins of Sympegmoid anatomy and reversions to C_3 photosynthesis (Figs. 2, 3).

2) *Haloxylon clade*—The *Haloxylon* clade is sister to the Sympegmoid clade in all shortest trees and includes *Haloxylon ammodendron, H. persicum, Girgensohnia oppositiflora,* and *Halothamnus subaphyllus.* The members of this clade have isopalisade anatomy with C_3 photosynthesis in cotyledons and Salsoloid Kranz anatomy, and except for *H. subaphyllus,* with hypodermis in green shoots/leaves. Botschantzev (1969, 1976) viewed *Haloxylon,* a genus of large shrubs, to be derived from section *Coccosalsola* subsection *Arbuscula,* and *Girgensohnia* (which are annuals) to be derived from annuals in section *Salsola.* Instead, however, the ITS phylogeny supports a sister-group relationship between *Haloxylon* and *Girgensohnia* (with 85% bootstrap support), and this clade is not closely related to either the *Arbuscula* or *Salsola* clades of the NADP-ME lineage (see below). *Halothamnus subaphyllus* is the sister to the *Haloxylon-Girgensohnia* sister pair in all shortest trees, although this relationship does not receive bootstrap support ≥50%. Based on photosynthesis types in assimilation organs (Table 1), *Halothamnus* is similar to the ''*Haloxylon*'' type, i.e., C_3 cotyledons with isopalisade mesophyll. Botschantzev (1969) considered *Halothamnus* to share a common ancestor with *Haloxylon,* a relationship generally supported by the shortest ITS trees, with the inclusion of *Girgensohnia* as the sister to *Haloxylon.* Some differences between *Halothamnus* and *Haloxylon* exist in cotyledon size, morphology, and longevity: cotyledons in *Haloxylon* are small (0.5–1 cm) and short-lived (~2 wk), whereas in *Halothamnus* they are longer (up to 1.5–2 cm) and longer-lived (~3–4 wk).

3) *Foliosa clade—Salsola zygophylla* and *S. foliosa* (the *Foliosa* clade) are the sister to the Sympegmoid + *Haloxylon* clade in all shortest trees, although bootstrap support for this relationship is <50%. These species are not part of the *Arbuscula* + *Salsola* clade (below), despite their biochemical and anatomical similarities (Table 1).

4) *Arbuscula and Salsola clades*—The *Arbuscula* and *Salsola* clades are sisters in all shortest trees (but with bootstrap support <50%) and contain species from section *Coccosalsola* subsection *Arbuscula* and section *Salsola,* respectively. The *Arbuscula* clade (*S. arbuscula, S. richteri, S. chiwensis,* and *S. angolensis*) and the *Salsola* clade (*S. kali, S. paulsenii,* and *S. australis*) are similar in photosynthesis type in leaves and cotyledons. The species of both clades have Salsoloid type Kranz anatomy and NADP-ME biochemistry in both cotyledons and leaves (Table 1). However, species of the *Salsola* clade lack hypodermal tissue in leaves, whereas hypodermis is present in species of the *Arbuscula* clade. The close relationships between these groups was also noted by Botschantzev (1969, 1976), who suggested a direct origin of section *Salsola* from subsection *Arbuscula.*

Evolutionary patterns in Salsoleae—Photosynthetic and anatomical characters have a dynamic history in Salsoleae, with C_3 photosynthesis in cotyledons and leaves, the development of hypodermis in mature leaves, and Sympegmoid leaf anatomy evolving independently in the NAD-ME and NADP-ME lineages. Although its history is less clear, cotyledon anatomy is quite diverse, with five anatomical types described for the species of Salsoleae included in the analysis; at least a few of these types likely evolved multiple times in parallel in Salsoleae.

Botschantzev (1969) proposed an elaborate hypothesis for the origin, diversification, and distribution of Salsoleae. He suggested that Salsoleae arose in Africa and subsequently dispersed to and diversified in the Middle East, central and eastern Asia, and Europe. Our reconstruction of geographic distributions contradicts this scenario. Instead, based on the species sampled, Salsoleae appear to have originated in central Asia, with more recent dispersal to Africa (independently for the ancestor of the *S. albisepala-S. albida* clade, *S. zygophylla,* and *S. angolensis*), to Mongolia (independently in the ancestor of *S. gemmascens* and *S. passerina* and in the broad-ranging *S. laricifolia, S. arbuscula, S. paulsenii, S. foliosa,* and *H. ammodendron*), and to Europe (e.g., *S. kali*) (Fig. 6). More species need to be collected from Africa for a more comprehensive evaluation of the origin of this tribe. In addition, the phylogenetic and biogeographical framework provided by this study should permit the development and testing of more informed hypotheses of the adaptations required for colonization and sur-

vival in the extremely harsh environments occupied by these plants.

Summary—This study demonstrates generally good agreement between clades inferred from phylogenetic analysis of ITS sequences (Fig. 1) and groups of species of *Salsola* based on morphological (e.g., Botschantzev, 1969, 1976) and physiological data (Table 1). Reconstruction of photosynthetic characters on the ITS phylogeny of Salsoleae demonstrates separate NAD-ME and NADP-ME lineages. The existence of two lineages of C_4 plants (NAD-ME vs. NADP-ME) in the tribe is further supported by analysis of a larger number of *Salsola* species based on structural and biochemical features of photosynthesis in leaves and cotyledons (Pyankov et al., 1997, 1998, 1999, unpublished data; Pyankov, Artyushera, and Edwards, 1999). The trees suggest a single origin of C_4 photosynthesis in Salsoleae (rather than independent origins of the two types from C_3 ancestors). It is thus possible that one type of C_4 photosynthesis evolved first and the other was derived from it, but this hypothesis will need to be evaluated by analysis of a larger collection of species in Salsoleae and other genera of Chenopodiaceae and Amaranthaceae. The clades within the NAD-ME and NADP-ME lineages generally share similar biochemical and anatomical characters of both leaves and cotyledons, although reversion to C_3 photosynthesis occurred in both clades. Reconstruction of geographic distributions suggests that Salsoleae originated and diversified in central Asia and subsequently dispersed to Africa, Europe, and Mongolia, in contrast to Botschantzev's (1969) hypotheses of biogeography and radiation.

LITERATURE CITED

AKHANI, H., P. TRIMBORN, AND H. ZIEGLER. 1997. Photosynthetic pathways in Chenopodiaceae from Africa, Asia and Europe with their ecological, phytogeographical and taxonomical importance. *Plant Systematics and Evolution* 206: 187–221.

ARTZICHOVSKII, V. M. 1928. Sexual growth and the development of its stem. *Trudy po Prirladnoi Botanike, Genetike I Selekzii.* 19 (in Russian).

BALDWIN, B. G., M. J. SANDERSON, J. M. PORTER, M. F. WOJCIECHOWSKI, C. S. CAMPBELL, AND M. J. DONOGHUE. 1995. The ITS region of nuclear ribosomal DNA: a variable source of evidence on angiosperm phylogeny. *Annals of the Missouri Botanical Garden* 82: 149–175.

BOTSCHANTZEV, V. P. 1967. *Sevadinae* Botsch., a new subtribe of the family Chenopodiaceae. *Botanicheskii Zhurnal* 50: 1053–1067 (in Russian).

———. 1968. A review of the species belonging to the section *Belanthera* Iljin of the genus Salsola L. *Botanicheskii Zhurnal* 10: 1440–1450 (in Russian).

———. 1969. The genus *Salsola* L., a concise history of its development and dispersal. *Botanicheskii Zhurnal* 54: 989–101 (in Russian).

———. 1974. Species of subsection *Caroxylon* of *Caroxylon* (Thunb.) Fenzl section of the genus *Salsola* L. *Novosti Sistematiki Vizshikh Rastenii* 11: 110–171 (in Russian).

———. 1976. Review of species *Coccosalsola* Fenzl section of genus *Salsola* L. *Novosti Sistematiki Vizshikh Rastenii* 13: 74–102 (in Russian).

———. 1979. Species subsection *Caroxylon* (Thunb.) Fenzl genus *Salsola* L. *Novosti Sistematiki Vizshikh Rastenii* 16: 110–171 (in Russian).

BUTNIK, A. A. 1979. Types of seedling development in Chenopodiaceae Vent. *Botanical Journal* 64: 834–842.

———, R. N. NIGMANOVA, S. A. PAIZIEVA, AND D. K. SAIDOV. 1991. Ecological anatomy of desert plants of Middle Asia. FAN, Tashkent, Russia (in Russian).

CAROLIN, R. C., S. W. L. JACOBS, AND M. VESK. 1975. Leaf structure in Chenopodiaceae. *Botanishe Jahrbucher fur Systematishe Pflanzengeschichte und Pflanzengeographie* 95: 226–255.

CULLINGS, K. W. 1992. Design and testing of a plant-specific PCR primer for ecological and evolutionary studies. *Molecular Ecology* 1: 233–240.

CZEREPANOV, S. K. 1995. Vascular plants of Russia and adjacent states (the former USSR). Cambridge University Press, Cambridge, UK.

DOWNIE, S. R., D. S. KATZ-DOWNIE, AND K.-J. CHO. 1997. Relationships in the Caryophyllales as suggested by phylogenetic analyses of partial chloroplast DNA ORF2280 homolog sequences. *American Journal of Botany* 84: 253–273.

DOYLE, J. J., AND J. L. DOYLE. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemistry Bulletin* 19: 11–15.

EDWARDS, G. E., AND M. S. B. KU. 1987. Biochemistry of C_3-C_4 intermediates. *In* M. D. Hatch and N. K. Boardman [eds.], The biochemistry of plants: a comprehensive treatise, vol. 14, Photosynthesis, 275–325. Academic Press, New York, New York, USA.

———, AND D. A. WALKER. 1983. C_3, C_4: mechanisms and cellular and environmental regulation of photosynthesis. Blackwell Scientific, Oxford, UK.

ELLIS, R. P. 1984. *Eragrostis walteri*—a first record of non-Kranz leaf anatomy in the subfamily Chloridoideae. *South African Journal of Botany* 3: 380–386.

FELSENSTEIN, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783–791.

FISHER, D. D., H. J. SCHENK, J. A. THORSON, AND W. R. FERREN, JR. 1997. Leaf anatomy and subgeneric affiliations of C_3 and C_4 species of *Suaeda* (Chenopodiaceae) in North America. *American Journal of Botany* 84: 1198–1210.

FREITAG, H. 1997. *Salsola* L. (Chenopodiaceae). *In* K. N. Rechninger [ed.], Flora Iranica, 154–255. Akademishe Druch- u Verlagsanstalt, Graz, Austria.

GAMALEY, Y. V., T. A. GLAGOLEVA, K. G. KOLCHESVSKI, AND M. V. CHLANOVSKAJA. 1992. Ecology and evolution of C_4-syndrome types in relation with phylogeny of families *Chenopodiaceae* and *Poaceae. Botanicheskii Zhurnal* 77: 2–12 (in Russian).

HATCH, M. D., AND T. KAGAWA. 1974. NAD malic enzyme in leaves with C_4-pathway photosynthesis and its role in C_4 acid decarboxylation. *Archives of Biochemistry and Biophysiology* 160: 346–349.

HATTERESLEY, H. A., AND L. WATSON. 1992. Diversification of photosynthesis. *In* G. P. Chapman [ed.], Grass evolution and domestication, 38–116. Cambridge University Press, Cambridge, UK.

KELLOGG, E. A. 1999. Phylogenetic aspects of the evolution of C_4 photosynthesis. *In* R. F. Sage and R. K. Monson [eds.], C_4 plant biology, 411–444. Academic Press, New York, New York, USA.

MADDISON, W. P., AND D. R. MADDISON. 1992. MacClade, version 3.04. Sinauer, Sunderland, Massachusetts, USA.

MANHART, J. R., AND J. H. RETTIG. 1994. Gene sequence data. *In* H.-D. Behnke and T. J. Mabry [eds.], Caryophyllales evolution and systematics, 235–246. Springer-Verlag, Berlin, Germany.

OLESEN, P. 1974. Leaf anatomy and ultrastructure of chloroplasts in *Salsola kali* L. as related to the C_4-pathway of photosynthesis. *Botaniska Notiser* 127: 352–363.

OSMOND, C. B. 1971. Metabolite transport in C_4 photosynthesis. *Australian Journal of Biological Science* 24: 159–163.

PYANKOV, V. I. 1991. The origin and evolution of C_4 metabolism of the Chenopodiaceae as a result of global aridization of climate. *In* Y. P. Abrol, P. N. Wattal, and A. Gnaham [eds.], Impact of global climatic changes on photosynthesis and plant productivity, 711–720. Oxford and IBH, New Delhi, India.

———, E. A. ARTYUSHEVA, AND G. E. EDWARDS. 1999. Formation of C_4-syndrome in leaves and cotyledons of *Kochia scoparia* and *Salsola collina*, Chenopodiaceae. *Russian Journal of Plant Physiology* 46: 527–542.

———, C. C. BLACK, E. G. ARTYUSHEVA, E. V. VOZNESENSKAYA, M. S. B. KU, AND G. E. EDWARDS. 1999. Features of photosynthesis in *Haloxylon* species of Chenopodiaceae that are dominant plants in Central Asia deserts. *Plant Cell Physiology* 40: 125–134.

———, A. N. KUZMIN, E. D. DEMIDOV, AND A. I. MASLOV. 1992. Diversity of biochemical pathways of CO_2 fixation in plants of the families *Poaceae* and Chenopodiaceae from arid zone of Central Asia. *Soviet Plant Physiology* 39: 411–420.

———, ———, M. S. B. KU, C. C. BLACK, E. G. ARTYUSHEVA, AND G. E. EDWARDS. 1998. Diversity of Kranz-anatomy and biochemical types of CO_2 fixation in leaves and cotyledons among Chenopodiaceae family. *In* G. Garab [ed.], Photosynthesis: mechanisms and effects, V, 4097–4100. Kluwer Academic Publishers, Dordrecht, The Netherlands.

———, AND D. V. VAKHRUSHEVA. 1989. The pathways of primary CO_2

fixation in Chenopodiaceae C_4-plants of Central Asian arid zone. *Soviet Plant Physiology* 36: 178–187.

———, E. V. VOZNESENSKAYA, A. V. KONDRATSCHUK, AND C. C. BLACK, JR. 1997. A comparative anatomical and biochemical analysis in *Salsola* (Chenopodiaceae) species with and without a Kranz type leaf anatomy: a possible reversion of C_3 to C_4 photosynthesis. *American Journal of Botany* 84: 597–606.

SHOMER-ILAN, A. S., A. NISSENBAUM, AND Y. WAISEL. 1981. Photosynthetic pathways and the ecological distribution of the Chenopodiaceae in Israel. *Oecologia* 48: 244–248.

SOLTIS, D. E., AND P. S. SOLTIS. 1997. Phylogenetic relationships among Saxifragaceae sensu lato: a comparison of topologies based on 18S rDNA and *rbcL* sequences. *American Journal of Botany* 84: 504–522.

SWOFFORD, D. L. 1998. PAUP*4.0: phylogenetic analysis using parsimony (and other methods), beta version 4.0. Sinauer, Sunderland, Massachusetts, USA.

TZVELEV, N. N. 1993. Notice about chenopods (Chenopodiaceae) of Eastern Europe. *Novi Taxoni* 50: 78–85 (in Russian).

VOZNESENSKAYA, E. V., AND Y. V. GAMALEY. 1986. The ultrastructural characteristics of leaf type with Kranz anatomy. *Botanicheskii Zhurnal* 71: 1291–1307 (in Russian).

WEN, J., AND E. A. ZIMMER. 1996. Phylogeny of *Panax* L. (the ginseng genus, Araliaceae): inferences from ITS sequences of nuclear ribosomal DNA. *Molecular Phylogenetics and Evolution* 6: 167–179.

ZALENSKII, O., AND T. A. GLAGOLEVA. 1981. Pathways of carbon metabolism in halophytic desert species from Chenopodiaceae. *Photosynthetica* 15: 244–255.